Please consider the Declaration and Power of Attorney signed by Mary MacDonald as the request under 37 CFR §1.42 to issue the patent to her as legal representative.

Claims 1-3 are rejected under 35 U.S.C. 103(a), as being unpatentable over Kermode and Ferry.

Claim 1 is rejected under 35 U.S.C. 103(a), as being unpatentable over Kermode and Ferry, *supra*, in view of Anderson.

Claims 1-3 are rejected under 35 U.S.C. 103(a), as being unpatentable over Kermode, Ferry, and Anderson as applied to claims 1-3 above, and further in view of Gleisner.

Claims 1, 4-8 are rejected under 35 U.S.C. 103(a), as being unpatentable over Kermode, Ferry, Anderson, and Gleisner as applied to claims 1-3 above, and further in view of Goodman and Gilman.

The four Section 103 rejections will be addressed in combination. Such a combined response is considered appropriate because *inter alia* each of the rejections relies upon at least the Kermode and Ferry as primary references.

Enclosed herewith is also a copy of the reference by Crowell et al. (Am. J. Physiol. 257: H107-H112, 1989) on which part of the response is based.

The examiner admits that the above references differ from the presently claimed invention by failing to explicitly disclose the use of formyl-Met peptides as pharmaceuticals. However, the examiner concludes that:

> [i]t would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to be motivated to make and use pharmaceutical compositions comprising formyl-Met peptides, in particular f-Met-Leu-Phe-Phe and f-Met-Leu-Tyr taught by Kermode and

> Ferry, because both references teach that formyl-Met peptides possess useful biological properties as they stimulate various functions of neutrophils which constitute defense reaction to infectious microorganismes. One would expect that *in vitro* observations of the effect of formyl-Met peptides on neutrophils will be translated into similar *in vivo* effect, because Kermode teaches that the rabbit peritoneal neutrophils is an adequate *in vitro* model as they have proved suitable for detailed biological characterization of the biological responses of neutrophils to chemotactic peptides.

Applicant respectfully disagrees. Each of the rejections is traversed.

The instant claims are drawn to pharmaceutical compositions comprising a formyl Met peptide having formula f-Met-Leu-X, wherein X is selected from the group consisting of Tyr, Tyr-Phe, Phe-Phe, and Phe-Tyr.

The Kermode reference is an attempt to prove the following mechanism by which formyl peptides stimulate neutrophil degranulation and chemotaxis (page 715, column 2, lines 3-15):

> One proposal for the neutrophil is that the **high-affinity form of the receptor** may be responsible for activation of some biological functions, notably chemotaxis, with the **low-affinity form** responsible for other functions, e.g. degranulation. Similar proposals have been made to explain the differential activation of a range of biological responses in several other cell types and with several other receptor agonists. The only evidence to date to support this hypothesis for the neutrophil, however, is derived from studies of the influence of various perturbations of the cell on both the receptor-binding pattern and the biological responses for a single chemotactic formyl peptide, the prototypical compound N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMet-Leu-Phe). [Emphasis added].

Kermode tests several different formyl peptides, including f-Met-Leu-Phe, and categorizes them into "most potent" and "less potent". Furthermore, Kermode concludes that the "most potent" peptides bind to the high affinity form of the

receptor, and that the "less potent" peptides bind to the low affinity form of the receptor. The "most potent" peptides according to Kermode are f-Met-Leu-Phe-Phe and f-Met-Leu-Phe-NHBzl. The "less potent" peptides are f-Met-Leu-Phe, f-Nle-Leu-Phe, f-Nva-Leu-Phe and f-Val-Leu-Phe. Thus, even amongst formyl peptides, according to Kermode, there are differences in potencies and in their mechanism of action.

Kermode, however, makes **no** suggestion for using formyl peptides for therapy or in a pharmaceutical composition. There is not even a hint of a suggestion by Kermode that such peptides will be useful for any therapeutic treatment.

In fact, Ferry teaches that administration of low molecular weight formyl peptides is **pro**inflammatory and, by whatever route, could cause **unwanted reactions or disorders**. For example, Ferry states on page 64, second column under Discussion:

> There is an increasing body of evidence suggesting that low molecular weight **proinflammatory** N-f-met oligopeptides **could play a role in intestinal inflammatory disorders**. All species of intestinal bacteria so far investigated produced such peptides in vitro and bioactive peptides have been demonstrated in colonic fluid obtained by in vivo dialysis techniques.
>
> In experimental animals **both colonic infusions and rectal administration of N-formyl methionyl-leucyl-phenylalanine (N-f-met-leu-phe) resulted in experimental colitis**, although the concentrations used in these studies were in the millimole range, at least three orders of magnitude greater than those estimated by bioassay of intestinal contents.
>
> **Systemic infusion** of radiolabeled f-met peptides in rats showed that intact peptide was rapidly excreted in bile and an enterohepatic circulation of f-met peptide was subsequently demonstrated. **Experimental acetic acid-induced colitis was associated with an eightfold increase in biliary excreation of labeled peptide following its instillation into colon loops**. [Emphasis added].

Ferry concludes from their own experimental data that (page 61, first column, lines 19-28):

> in the ileum both enzymic degradation and restricted mucosal permeability contribute to the intestinal barrier to luminal bacterial formyl oligopeptides. In the colon, however, enzymic mechanisms are less active and restricted mucosal permeability is the major factor. **Abnormalities of the intestinal mucosal barrier to proinflammatory bacterial peptides could play a role in inflammatory disorders of the gut**.

Although their conclusion focuses on administration of formyl peptides to the **unhealthy** intestine, they also suggest problems even if administered to **healthy** individuals. Ferry admits that their failure to find increased absorption to the intestine under normal conditions cannot in any way be used even to assume, much less to predict with reasonable certainty, that these peptides will have no adverse effect when administered to healthy individuals (paragraph bridging pages 65-66):

> **Changes in vascular permeability and blood flow (without changes in mucosal permeability) have been reported with f-met-leu-phe in rat small intestine** by Granger et al. and these effects were apparently not found in animals rendered neutropenic, suggesting an effect of f-met-leu-phe on neutrophil leukocytes in the microcirculation of the gut. More recently, **the same group reported increased mucosal permeability in response to ileal perfusion with f-met-leu-phe (10^{-6}M)**. This observation supports that of Magnussen et al. The effect appears to be confined to the terminal ileum and to be leukocyte-dependent. **We failed to find increased ^{51}Cr-EDTA absorption with either f-met-leu-tyr (10^{-4}M) or f-met-leu-phe (10^{-4}M) alone over a 1-h period. The short period of observation and the infusion into loops rather than perfusion design may account for this. Our studies were simply designed as controls for our experiments with different agents rather than to investigate the inflammatory response and permeability changes secondary to leukocyte accumulation. Trace amounts of intact formyl peptides do escape the enzyme and mucosal permeability barriers and trace amounts of intact peptide (picomoles) were recovered in bile in our control studies**. The biological significance of these amounts awaits further studies. [Emphasis added].

Ferry suggests that the trace amounts of formyl peptide in bile **may be** a symptom of potential adverse effects even under healthy conditions but has not investigated this issue. However, based on the wealth of information provided by others, one of ordinary skill in the art would consider it is likely.

Thus, both Kermode and Ferry **fail** to teach that the formyl peptides of the instant invention would be useful as pharmaceutical compounds. In fact, they **teach away** from their use.

For example, Kermode teaches that (page 1991, right column):

> [t]he logical interpretation of these data is thus that the high-affinity sites are the receptors that initiate degranulation.

Because Kermode also teaches that f-Met-Leu-Phe-Phe binds to the high affinity receptor, one skilled in the art would be expected to conclude that f-Met-Leu-Phe-Phe initiates degranulation of the neutrophils and thus is harmful.

Earlier Kermode postulated that the high affinity site was responsible for activiating chemotaxis, which also is harmful. Thus, it is not seen how any teaching of Kermode would lead one of ordinary skill in the art to make a pharmaceutical composition as claimed herein. Indeed, the first discovery that the claimed f-Met peptides provide useful biological properties was made by Applicant. Indeed, this useful property has been found only in the few claimed peptides, not in all f-Met peptides.

Ferry supports Kermode in teaching that f-Met peptides cause harmful effects. In view of these teachings, why would one of ordinary skill in the art even consider the use of f-Met peptides in a pharmaceutical composition. Indeed, althought there are extensive publications relating to f-Met peptides, to Applicant's knowledge, none

of them suggest administering such peptides for any beneficial effect.

Anderson does not make up for any of the deficiencies of Kermode and Ferry. The studies of Anderson also support the notion that formyl peptides and their analogues may **cause inflammatory disorders** and thus **would not be useful** as pharmaceutical compounds. For example, Anderson first recites the types of disorders that may be associated with formyl peptides and then suggests a mechanism for the cause of such disorders (page 249, first column, lines 1-10; page 254, second column, lines 32-41):

> **There is now a substantial body of evidence implicating bacterial F-met peptides in intestinal inflammatory disorders**. They induce adhesion, chemotaxis, superoxide production, and lysosomal enzyme release in neutrophil leukocytes; **can induce experimental colitis** in mice, rats, and rabbits; **increase intestinal vascular and mucosal permeability**; stimulate intestinal leukotriene synthesis; and are **spasmogenic for gut smooth muscle**.***
>
> Using a radioimmune assay with a rabbit polyclonal antibody raised against FMLP, we have identified FMLP immunoreactivity in both rat and human bile. The most likely source of this reactivity is formyl oligopeptide produced by intestinal bacteria and reaching the liver in portal blood. **Since the liver excretes such peptides in a largely unaltered form, they presumably retain their potential to induce inflammatory responses should they cross the biliary epithelium**. [Emphasis added].

Anderson concludes that (page 255, column 1):

> The association between biliary tract disorders and inflammatory bowel disease has long been thought to be related to the presence of bacterial products in bile, **and low-molecular-weight formyl-peptides could be important in this respect**. [Emphasis added].

Thus, the Anderson reference also **teaches away** from using formyl peptides and their analogues as pharmaceutical compositions.

Gleisner also fails to make up for the deficiencies of the above discussed

references. Gleisner suggests that f-Met-Leu-Phe can have an inhibiting effect on mast cell degranulation but fails to show this for other formyl peptides. It is **not** obvious that structurally similar compounds will have the same effect **or** the same potency, if at all, and this is supported by the study of Kermode, where they observed differences in potency of various formyl peptides. In this respect, the potency of activation of neutrophils by various peptides described in Kermode **cannot** be used to correlate the effect on the inhibition of mast cells. In fact, Gleisner admits that they have not tested other formyl peptides for inhibition potential (page 16, lines 6-8):

> In this report we have **not** considered in detail the relative potencies of f-methionyl peptides and pepstatin because the blueing reaction is only semiquantitative. [Emphasis added].

Thus, given the many teachings that f-met peptides are harmful and corresponding the lack of incentive to administer the f-Met peptides for therapeutic effect, one of ordinary skill in the art would not have been motivated to make and use a pharmaceutical compound for the formyl peptides of the present invention. As noted by Kermode (as cited above), local and systemic administration of f-Met peptides have both been associated **to induce** intestinal inflammatory disorders. Thus, the lack of motivation to develop and use pharmaceutical compounds of f-Met peptides encompasses all routes of administration. It makes moot the rejection relying upon Goodman and Gilman.

In addition, it is also suggested by the reference of Crowell et al., *Am. J. Physiol.* 257 (*Heart Circ. Physiol.* 26): H107-H112, 1989, a copy of which is enclosed herewith, that f-Met-Leu-Phe ("FMLP") may indeed have adverse effects, **even in the context of a normal immune response to bacterial infection**.

In the introductory paragraph, Crowell states that FMLP has been shown to possess spasmogenic properties in smoothe muscle preparations from various organs.

In column 2 (H107), Crowell states that "FMLP has been shown to cause systemic hypertension, presumably through vasoconstriction . . ." On page H111, Crowell states that (lines 30-40):

> Although the clinical significance of formylated peptides is not known, **increased pulmonary vascular pressures are observed** in animals injected intravenously with bacteria capable of producing these substances. Such a response is a recognized component of the clinical pulmonary vascular response to **bacteremia**. If these substances are released by microorganisms in vivo, formylated peptides such as **FMLP may play a significant role in the pulmonary vascular changes observed in this disease**. [Emphasis added].

In sum, it appears that the inflammatory response that had previously been thought of as a protective mechanism from bacteremia, may actually be involved in **worsening the situation with hypoxemia, vasoconstriction, and systemic hypertension** (page H107, column 2, lines 2-9), with FMLP playing a significant role.

In view of the prior art of record, it is apparent that one of ordinary skill in the art would have considerable doubt on the usefulness of FMLP in therapy or in a pharmaceutical composition based on the above evidence and argument.

Only Applicant has discovered the usefulness of the claimed invention in a pharmaceutical composition. Further, as shown in Table 1, many formyl peptides have no effect on inhibition of mast cell degranulation. FMLP has an effect of 30% inhibition of mast cell degranulation. Surprisingly and unexpectedly, the compounds of the present invention have an effect of 55% or more inhibition of mast cell degranulation with the preferred f-Met-Leu-Phe-Phe illustrating 100% inhibition of mast cell degranulation.

In view of the above, it is not seen how the present invention would have been obvious to one of ordinary skill in the art. Reconsideration and withdrawal of the rejections are requested.

It is respectfully submitted that the present application is in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

Respectfully submitted,

9/1/99

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Responses of isolated pulmonary arteries to synthetic peptide F-Met-Leu-Phe

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CROWELL, RICHARD E., DENNIS E. VAN EPPS, AND WILLIAM P. REED. *Responses of isolated pulmonary arteries to synthetic peptide F-Met-Leu-Phe.* Am. J. Physiol. 257 (Heart Circ. Physiol. 26): H107–H112, 1989.—The chemoattractant formyl-methionine-leucine-phenylalanine (FMLP) has recently been shown to possess spasmogenic properties in smooth muscle preparations from various organs. In this study we have investigated the actions of this peptide on isolated rabbit pulmonary artery (PA) ring segments. FMLP stimulated concentration-dependent constriction of PA at resting tension. However, in PA that had been preconstricted by norepinephrine, FMLP stimulated concentration-dependent relaxation. FMLP-stimulated PA constriction was inhibited by earlier exposure to indomethacin or to furegrelate, a thromboxane synthetase inhibitor, but not by earlier exposure to the H1 histamine receptor antagonist pyrilamine. FMLP-stimulated relaxation of PA was totally abolished by indomethacin but not by furegrelate or pyrilamine. Disruption of the endothelium in PA preparations decreased both the constriction and relaxation response to the peptide, suggesting that these cells were involved in these responses. These results indicate that the chemotactic factor FMLP can elicit constriction or relaxation of isolated PA, depending on the underlying active PA tension. In addition, both constriction and relaxation are dependent on cyclooxygenase products and intact endothelium.

rabbit pulmonary arteries; formyl-peptide-induced vasoconstriction; formyl-peptide-induced vasodilation

CHEMOTACTIC FACTORS such as the complement component C5a and the synthetic peptide formyl-methionine-leucine-phenylalanine (FMLP) stimulate the directional locomotion of leukocytes and play an important role in the inflammatory response. Interaction of these factors with specific receptors on neutrophils and monocytes not only induces chemotaxis in these cells but also stimulates aggregation, enzyme secretion, and oxidative metabolism (29). C5a and FMLP also possess potent smooth muscle spasmogenic activity in many tissues including ileum, bronchus, and lung parenchymal strips (3, 6, 12, 20, 30).

In addition, C5a possesses potent vasoactivity in several vascular beds including pulmonary arteries (PA; 15, 21). These data indicate that the role of C5a in the inflammatory response includes modulation of the vascular bed. Such C5a activity in the lung could cause abnormal regulation of pulmonary vascular tone in areas of complement activation, leading to mismatching of ventilation and perfusion within the lung and subsequent hypoxemia. FMLP has been shown to cause systemic hypertension, presumably through vasoconstriction (12); however, direct effects of this peptide on vascular tissue are unknown. If such effects can be demonstrated in pulmonary vascular tissue, it may suggest a role for formyl peptides in the development of hypoxemia in acute pulmonary inflammatory responses to bacterial infection.

Although the actions of C5a and FMLP on tissues containing smooth muscle have been described, the mechanism of peptide action in these tissues is not understood. In some species, the contractile action of C5a is suppressed by cyclooxygenase and lipoxygenase inhibitors (21, 24). However, these pharmacological agents have varied effects on FMLP-induced contraction of human airway and guinea pig lung preparations (3, 12). In addition, although the action of these peptides is likely mediated via interaction with specific receptors (20), the cellular distribution of these receptors is unknown. This study was designed to investigate: *1*) the effects of FMLP exposure on isolated rabbit PA; *2*) the involvement of cyclooxygenase products, including thromboxane, through the use of pharmacological inhibitors; and *3*) the role of endothelium in PA responses to FMLP.

METHODS

Experimental model. New Zealand White rabbits of either sex weighing from 2.5 to 3 kg were killed, an anterior median sternotomy performed, and the entire contents of the thoracic cavity removed and placed in modified Tyrode solution (MTS). The MTS was composed of (mM /l): 130 NaCl, 2.5 $CaCl_2$, 4.0 KCl, 1.0 NaCl, 35 NaH_2PO_4, 10.0 $NaHCO_3$, and 5.56 dextrose. After repeated washing, the lungs, heart, and connecting blood vessels were placed in a dissecting dish containing MTS, and one of the major PA was selected and dissected free from surrounding lung and connective tissue from the division of the main PA through the first lobar branch. Ring segments from 2 to 4 mm in length were excised from the lobar region. Excessive manipulation of the tissues was avoided to minimize endothelial cell damage. Each ring segment was then attached to a Grass FT 03 force displacement transducer via stainless steel hooks that were gently placed into the lumen of each ring segment. The segment was then suspended in a 12-ml

vertical tissue bath filled with MTS and aerated continuously with 98% O_2-2% CO_2 so that pH was maintained at ~7.4 for the entire experiment. The ring segments were allowed to stabilize (no change in base-line force for 30 min) at 36.5°C. PA were stabilized at a base-line force of 2–2.5 g. This force is ~50–75% of that required for optimal response to standard agonists (5 μM norepinephrine, 100 μM histamine) as determined by other investigators (1, 14) and in preliminary experiments in our laboratory.

Vasoactive substances were added directly to the muscle bath. The force developed in response to a substance added to the bath was noted as the change of force from base line (g). Tension (g/mm^2) was then calculated by dividing the change in force by the cross-sectional area. The cross-sectional area (mm^2) of the vascular wall perpendicular to the direction of generated force was determined by assuming a tissue specific gravity of unity and dividing the wet weight of each tissue segment (determined after gentle blotting) by the width of the vessel between the stainless steel loops that support the tissue within the chamber. All PA responses to FMLP are expressed as a percentage of the maximum PA response to a reference agonist administered to the tissue either immediately before or after the peptide. Reference agonists used included 100 μM histamine or 5 μM norepinephrine. Preliminary experiments demonstrated that PA responses to each agonist at these concentrations were the same.

FMLP stimulation of PA. After PA segments were appropriately prepared, FMLP was added directly to the tissue bath, the maximal response noted, and the bath solution replaced repeatedly until the PA response returned to base line. Because of previous reports of prolonged tissue desensitization following repeat applications of other chemotactic factors (6, 21) and our own preliminary studies, dose-response curves were performed using only a single exposure of PA to FMLP. Desensitization experiments were performed by reexposing a single tissue to the peptide at varying intervals after the first application.

Studies with pharmacological antagonists. In experiments requiring the addition of pharmacological agents, paired ring segments were harvested from adjacent portions of the same PA. An inhibitor was added to one bath, whereas the other segment served as a control. FMLP was then added to both tissue baths, and the PA response was noted. The responses of the paired PA segments were then compared. None of the solvents used to dissolve the inhibitors (maximum volume of solvent added to bath was 25 μl) affected the tissue preparations.

Disruption of PA endothelium. After an initial challenge of the PA with NE, the endothelium was disrupted by gentle rubbing with a stainless steel dissecting probe, being careful not to stretch the tissue. This procedure is similar to a method of endothelial disruption previously described by Furchgott et al. (10). Other investigators have demonstrated histologically that this method effectively disrupts the entire endothelial surface while leaving the underlying smooth muscle layers intact (9). The bath was then changed twice to eliminate any soluble materials released by the damaged endothelial cells, and the PA segment was allowed to rest undisturbed for 60 min before rechallenge with NE. PA segments exhibiting <90% of the initial response to NE were discarded because of possible damage to the contractile apparatus of the tissue. Endothelial disruption was confirmed by the addition of 100 nM acetylcholine (ACh) after NE preconstriction. Preliminary studies demonstrated that this concentration of ACh induces relaxation of preconstricted PA with intact endothelium but contracts PA after endothelial disruption.

Statistical analysis. Significant differences in responses of paired PA segments were determined using the paired *t* test. Data are presented as means ± SE except where noted.

Reagents. NE, pyrilamine, indomethacin, and FMLP were obtained from the Sigma Chemical (St. Louis, MO). Furegrelate was a generous gift from UpJohn, Kalamazoo, MI. Stock solutions were prepared by dissolving NE in MTS, pyrilamine and furegrelate in distilled water, indomethacin in 95% ethanol, and FMLP in dimethyl sulfoxide. Small volumes of these solutions were then added directly into the tissue baths. All solutions were made fresh daily and protected from light.

RESULTS

PA responses to FMLP. PA exhibited distinct responses to FMLP under different experimental conditions. FMLP stimulated an immediate and rapid contraction when applied to PA at resting base-line tension. As shown in Fig. 1, threshold concentration for FMLP-stimulated PA contraction was ~1 nM and reached a plateau at 100 nM. After reaching maximal contraction, the PA segment rapidly relaxed toward the original base-line tension. Maximal PA contraction was reached within 60 s after FMLP exposure with subsequent relaxation lasting another 2–3 min.

Although FMLP contracted PA at resting tension, application of 10 nM or less of the peptide to PA preconstricted with 5 μM NE resulted in tissue relaxation. Responses of preconstricted PA to FMLP were also

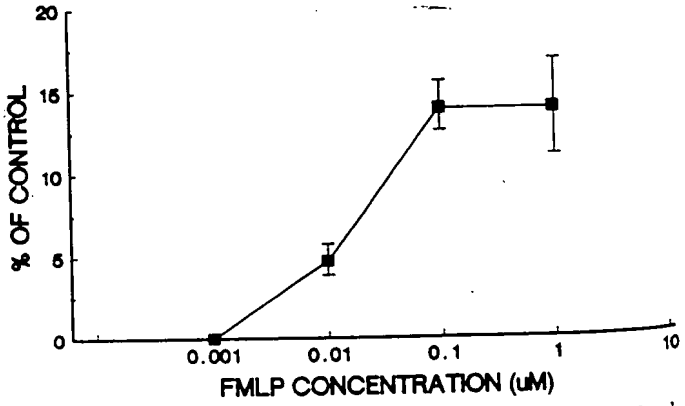

FIG. 1. Dose-response curve for FMLP-induced contraction of pulmonary artery (PA) ring segments at resting tension expressed as means ± SE percentage PA response to FMLP compared with maximal PA constriction in response to either 5 μM norepinephrine or 100 μM histamine. PA contractile responses to agonists at these concentrations were same. Each point represents mean of 4 different PA preparations.

concentration dependent (Fig. 2). Exposure of preconstricted PA to 1 nM of FMLP resulted in PA relaxation of ~3%; 10 nM of FMLP caused 15% relaxation or about half the magnitude of relaxation caused by a maximally relaxing concentration of 100 nM ACh. Most (but not all) PA responses to FMLP at these concentrations were preceded by a small, rapid contraction before the more prolonged relaxation phase. FMLP-induced relaxation was observed only after PA preconstriction. At concentrations of 100 nM, the FMLP caused further PA contraction that averaged ~7% above the preconstricted PA tension. However, preconstricted PA responses to this peptide concentration were sluggish and somewhat variable. Two segments exhibited no significant response to the peptide at this concentration, but three others displayed a slow, sustained contraction to a new peak tension ~10% higher than that achieved by NE preconstriction. This slow contractile response occurred after a lag period of ~30 s and was in contrast to the immediate and rapid contraction observed at resting base-line PA tensions. Relaxation of preconstricted PA was never observed at this concentration of FMLP. Resting PA and preconstricted PA remained desensitized to repeat application of FMLP for at least 60 min after exposure to the peptide (data not shown).

Effects of histamine, cyclooxygenase, and thromboxane antagonists on FMLP-stimulated responses. Pyrilamine was used to determine whether the H1 histamine receptor was involved in the PA response to FMLP. Although 10 μM pyrilamine completely inhibited histamine actions on PA, it had no effect on FMLP-induced PA contraction or relaxation (Fig. 3). Indomethacin, a cyclooxygenase inhibitor, was used to examine the possible role of products of this pathway of arachidonic acid metabolism. At a concentration that is an effective inhibitor of cyclooxygenase activity (1 μM; 16, 32), indomethacin had no effect on PA responses to histamine or NE but nearly eliminated PA constriction stimulated by FMLP, decreasing the response by ~93% (see Fig. 3).

Thromboxane is a major metabolite of cyclooxygenase and has been implicated as a cause of pulmonary vasoconstriction after systemic complement activation in vivo (8, 17, 33). To test if this mediator was involved in the PA response to FMLP, the thromboxane synthetase inhibitor furegrelate (11) was placed in the bath before the addition of FMLP. Furegrelate (1 μM) significantly decreased peptide-induced contraction of PA at resting tensions (Fig. 3). As noted in Table 1, furegrelate tended to decrease FMLP-induced relaxation of preconstricted PA, but this effect was not significant ($P = 0.35$). Although relaxation was not eliminated, the early small contraction observed before relaxation did not occur when furegrelate was added before PA preconstriction. Furegrelate did not affect PA responses to NE (Fig. 3). Furegrelate effects on PA contraction by histamine were not evaluated.

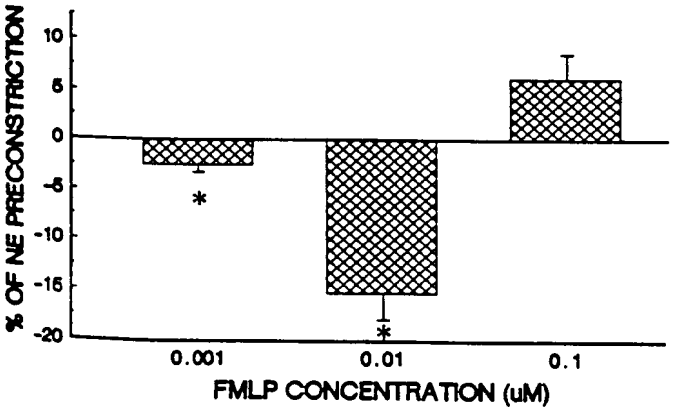

FIG. 2. Concentration-response relationships for FMLP actions on preconstricted pulmonary artery (PA) segments expressed as means ± SE percentage change in PA tension from maximal PA preconstriction by 5 μM norepinephrine (NE). A positive percent change represents further PA contraction, whereas a negative percent change represents PA relaxation. Each bar represents mean of 5 different PA preparations. * $P < 0.05$.

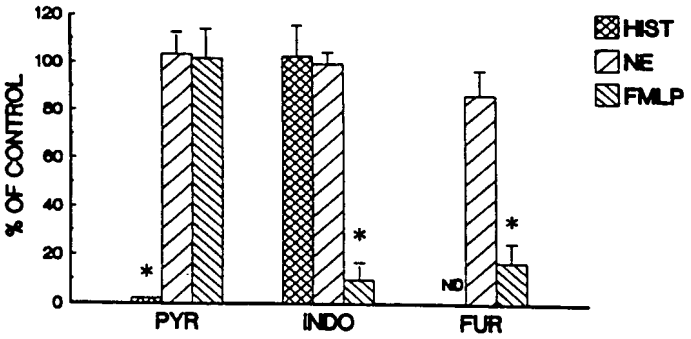

FIG. 3. Effects of pyrilamine (PYR), indomethacin (INDO), and furegrelate (FUR) on PA contraction stimulated by 100 nM FMLP, 100 μM histamine (HIST), and 5 μM norepinephrine (NE). Each bar represents means ± SE percent contraction of PA exposed to each inhibitor compared with controls. Data represents means of 5 different PA preparations. * $P < 0.05$; ND, not done.

TABLE 1. *Effect of indomethacin and furegrelate on FMLP-induced relaxation of preconstricted PA*

	FMLP (10^{-8} M)	FMLP + Indomethacin	FMLP + Furegrelate
% Relaxation from peak NE response	15.3±2.8	0*	8.8±3.1

Values are means ± SE; n, 4 segments. PA, pulmonary artery; NE, norepinephrine. * $P < 0.01$ for comparison with FMLP alone.

Indomethacin also modified the actions of FMLP on preconstricted PA. Addition of 1 μM of indomethacin to the bath before NE abolished the relaxation of preconstricted PA exposed to 10 nM of the peptide (Table 1). In the presence of indomethacin, the relaxation response of PA to these peptide concentrations was replaced in some PA segments by a delayed, slow, sustained increase in tension similar to that observed at higher concentrations of FMLP. Thus cyclooxygenase inhibition modulated two of the three PA responses to FMLP previously described.

Role of endothelium in FMLP-stimulated PA responses. Two sets of experiments were performed to investigate the role of endothelium in the actions of FMLP on isolated PA. The initial experiments were designed to determine the role of endothelium on FMLP-stimulated constriction at resting tensions. As shown in Fig. 4*A*, endothelial disruption significantly decreased the contractile response of resting PA to 100 nM FMLP ($P < 0.05$); however, the peptide still stimulated a rapid con-

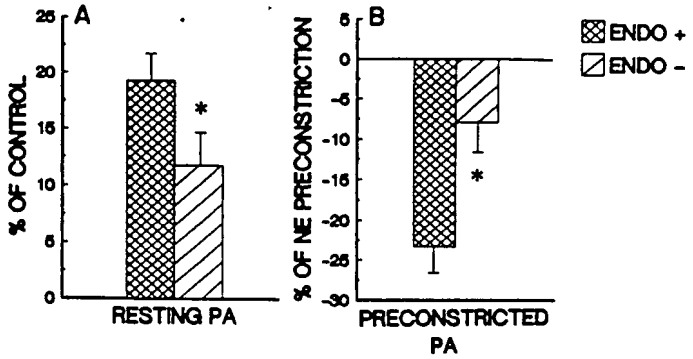

FIG. 4. Effects of endothelial cell disruption on FMLP-induced contraction of resting pulmonary artery (PA) (*A*) and relaxation of preconstricted PA (*B*). ENDO+, PA with intact endothelium; ENDO−, PA with disrupted endothelium. *A*: mean percent PA contraction by 100 nM FMLP (±SE) for 4 different PA preparations at resting tension compared with a reference contraction by 100 μM histamine. PA constriction by 100 μM histamine was equal to constriction by 5 μM norepinephrine (NE). *B*: mean percent relaxation of preconstricted PA by 10 nM FMLP (±SE) for 4 different PA preparations. * $P < 0.05$.

tractile response followed by rapid relaxation in the denuded tissues. The second set of experiments were designed to determine the role of endothelium on FMLP-stimulated relaxation responses in PA preconstricted with NE (Fig. 4*B*). Although endothelial disruption did not totally abolish the relaxing actions of FMLP on preconstricted PA, the procedure did significantly attenuate the effect ($P < 0.05$). These results provide evidence that both the contractile and relaxing actions of FMLP in PA under different experimental conditions may be mediated in part by endothelium.

DISCUSSION

FMLP is a member of a family of synthetic bioactive peptides that exhibit a broad range of actions in phagocytic cells (29). Recently, this peptide has been shown to possess spasmogenic activity in vitro in a number of smooth muscle preparations (3, 12, 20). This study demonstrated that FMLP also has myotropic actions on isolated PA ring segments. In addition, we determined that this peptide is capable of eliciting both contraction and relaxation in these preparations. The nature (contraction vs. relaxation) and degree of PA responsiveness is dependent on several factors, including degree of underlying tension of the PA preparation, peptide concentration, generation of cyclooxygenase products of arachidonic acid metabolism, and the presence of intact endothelium on the preparation.

The heterogeneity of vascular responses to FMLP observed in our experiments has been shown with other vasoactive substances. Concentration dependence is characteristic of vasoactive substances and has been observed in agents that contract as well as relax vascular smooth muscle. In addition, several vasoactive agents are capable of eliciting contraction as well as relaxation, depending on experimental conditions. Substance P contracts isolated PA segments at resting tensions but induces relaxation after preconstriction of the vessels (31). Although NE is usually considered a potent vasoconstrictor, it is also capable of relaxing isolated vessels from certain vascular beds (7, 26). This variability of action exhibited by NE is mediated by stimulation of different and separate receptor populations, each population generating a different tissue response (26). As it is likely that PA responses to FMLP are also receptor mediated; one explanation for the heterogeneity in PA responsiveness to this peptide in our experiments is the existence of distinct FMLP receptor types within the tissue that mediate different responses to the peptide.

However, studies of FMLP receptors in neutrophils indicate the presence of only one specific receptor for this peptide (34). This receptor exists in two different affinity states, each of which is associated with distinct biological functions (19). It is possible that active vascular smooth muscle contraction before FMLP exposure alters cell membranes and results in modification of the tissue response to FMLP. Such a phenomenon has been shown in neutrophils where exposure of these cells to different peptide concentrations alters the affinity of the receptor for ligand and also changes the response of the cell. For example, although progressively higher FMLP concentrations generally increase chemotactic responsiveness of neutrophils to this peptide, once concentrations >100 nM are reached, chemotaxis is reduced; however, at concentrations of 100 nM or greater, neutrophils respond to FMLP by producing superoxide anion, which does not occur at lower concentrations. This change in function parallels a change in the proportion of high to low affinity receptors on the cell membrane (29). Such concentration effects on receptor affinity and function may also account for the change in preconstricted PA response at 100 nM from relaxation to contraction.

PA responses to FMLP were modulated not only by changing peptide concentration or the degree of underlying active PA tension but also by inhibition of cyclooxygenase. Although experiments using pharmacological inhibitors are not definitive because of the relative degree of specificity and multiple actions of these agents, our experiments do suggest that prostanoids play a role in the FMLP effects on isolated PA. It is known that lung vascular tissue is capable of producing a variety of vasoactive substances via cyclooxygenase metabolism (17, 18, 27). Such substances have been shown to be generated by isolated rabbit PA following exposure to C5a (15) and by the pulmonary vascular bed in vivo after intravenous endotoxin (17, 33). In addition, other studies have demonstrated that cyclooxygenase products are involved in FMLP-induced constriction of isolated parenchymal lung strips (12).

PA responses to FMLP were also partially dependent on the presence of intact endothelial cells. It is well known that these cells modulate smooth muscle contractility in vessels from several different vascular beds, including pulmonary arteries (2, 9, 22, 31). NE, substance P, acetylcholine, bradykinin, and thrombin all induce endothelial-dependent relaxation of preconstricted arterial segments in certain species (2, 7, 9, 10, 31). Our data indicate that endothelial cells not only modulate FMLP-induced relaxation of preconstricted PA but also facilitate contraction of PA by the peptide at resting tension. Agents exhibiting endothelial-dependent contraction of vascular tissue have been described (9, 13, 22), and

cultured endothelial cells have been shown to release a potent constrictor of pulmonary arteries (23). Therefore, our findings suggest that these cells release both contractile and relaxant vasoactive substances as a result of interaction of FMLP with the vessel wall. Other investigators have suggested that endothelium possesses receptors for formylated peptides (25). Although it is possible that this interaction is mediated through receptors on the endothelial cell surface, we cannot conclude from our studies whether FMLP acts directly on endothelium or indirectly via a factor or factors released from other cells within the vascular wall.

The experiments described here indicate that the synthetic peptide FMLP invokes complex responses in isolated PA ring segments. It is conceivable that responses of larger PA vessels to FMLP may not be representative of the smaller arterioles and microvasculature of the lung. However, there is evidence that acetylcholine, another vasoactive agent with heterogeneous actions on large PA, induces endothelial-dependent vasodilation in isolated rabbit lung preparations (5). Such data suggest that acetylcholine acts via the endothelium of resistance vessels to elicit vasodilation. Experiments showing that endotoxin-mediated pulmonary vasoconstriction is dependent on cyclooxygenase products suggests that these substances also act on resistance vessels within the lung (17, 33). Since the actions of FMLP are dependent on both cyclooxygenase products and the presence of intact endothelium for maximal effect, it is possible that FMLP acts on resistance vessels in a similar manner. Although the clinical significance of formylated peptides is not known, increased pulmonary vascular pressures are observed in animals injected intravenously with bacteria capable of producing these substances (4). Such a response is a recognized component of the clinical pulmonary vascular response to bacteremia (28). If these substances are released by microorganisms in vivo, formylated peptides such as FMLP may play a significant role in the pulmonary vascular changes observed in this disease.

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